

IN THE UNITED STATES PATENT & TRADEMARK OFFICE	
IN RE APPLICATION OF:  SENN ET AL.	
APPLICATION NO.: 10/801,405	
Filed: March 16, 2004	Group Art Unit: 1616
For: Pesticidal Compositions	Examiner: PRYOR, ALTON M.
Commissioner For Patents P.O. Box 1450 Alexandria, VA 22313-1450	

**DECLARATION UNDER RULE 132**

I, Elke Hillesheim, a citizen of Germany, residing in Basel, Switzerland, hereby declare:

**CREDENTIALS**

**My Experience**

- Project Biologist / Insecticides (SYNGENTA CROP PROTECTION AG)
  - January 2003 until Present
- Team Leader Micro Screens / Insecticides (SYNGENTA CROP PROTECTION AG)
  - January 2001 until January 2003
- Laboratory Leader in HTS-O / Insect Control (NOVARTIS CROP PROTECTION)
  - August 1996 until December 2000
- Laboratory Leader in Research Group Plant Physiology / Biochemistry (SANDOZ AGRO)
  - January 1996 until September 1996
- Laboratory Leader in Entomology (SANDOZ AGRO)
  - Aug. 1991 until Dec. 1995

**My Appointments and University Positions**

- 1991 Scientific associate at the Centre of Teaching and Research (ZLF) in Basel
- 1988 – 1990 Post-Doctorate position at the Zoological Institute of the University of Basel
  - Reaction norms of *Drosophila melanogaster*.

- 1987 – 1988 Scientific associate at the Institute for honeybee breeding in Erlangen
  - Kin-recognition among honeybees detected by oxygen consumption.
- 1983 – 1987 Scientific associate at the University of Frankfurt a.M.
  - Projects:
    - Heritability of physiological and ethological characteristics of the honeybee.
    - Genetically determined dominance of worker bees and its influence on the performance of the colony.

### **My Education and Training**

- 1987 Dissertation: “Individual Dominance and its influence on performance of the colony of *Apis mellifera capensis* ESCH.”
- 1983 Diploma in biology
- 1978 – 1983 Study of Biology at the J.-W. Goethe Universität in Frankfurt a.M.
  - Major subject: Zoology
  - Minor subjects: Biochemistry, Botany, Pharmacology
- 1966 – 1978 University entrance diploma at ‘Staatliches Neusprachliches Gymnasium in Mayen’ (D)

### **My Awards Received**

- 2006: Syngenta Local Stein Award (1<sup>st</sup> prize/Category technology): Establishment of Micro Profiling Screens – Evolution of a modern screening platform
- 1999: Idea Nova Global Award (2<sup>nd</sup> prize): High Throughput Screening on Target Organisms

### **My Areas of Expertise:**

- Entomology / Plant protection / Population genetics
- Project-Management
- Anti-resistance-Projects
- GLP experience for bee toxicity studies
- Field trials – Organization, Analysis, Interpretation, Presentation
- Development, validation of various test methods
- Development and establishing of an HTScreening (Entomology)
- Establishment of special tests (Entomology, Herbology)
- Experience in biochemical tests (ELISA, protein determination)

## **COMPARATIVE PROCEDURES**

1. That the following tests were carried out under my supervision in a Laboratory in Stein / Aargau/Switzerland to determine if mixtures of Abamectin and Thiamethoxam have synergistic effects on:
  - a. *Heliothis virescens*
    - i. Tobacco budworm
    - ii. Ovicidal (eggs and L1)
  - b. *Plutella xylostella*
    - i. Diamond-back moth (L2)
  - c. *Tetranychus urticae*
    - i. Two-spotted spider mite (mixed population)
2. The following bioassays were performed:
  - a. *Heliothis virescens* (MPS Method)
    - i. 24 well microtiterplates (MTP) were used. Each well contained 0.5 ml *Heliothis* diet. Each well contained 30 to 40 eggs. The test solution was pipetted on top of the eggs and the artificial diet (40 µl per well). 24 hours after the application the MTP's were covered with a thick filter paper and a stainless steel lid containing holes. The MTP's were incubated in an incubator at 28/27 °C at 60 % relative humidity with 14 hrs of light. Assessment was made 4 days after application. Ovicidal activity and % mortality was assessed on first instars. 6 replicates per concentration per product.
  - b. *Plutella xylostella* (MPS Method)
    - i. 24 well microtiterplates (MTP) were used. Each well contained 0.5 ml *Plutella* diet. 50 µl test solution was pipetted on top of the diet. 24 hours later 10 larvae (L2) were placed in each well. The MTP's were covered with a thick filter paper and a stainless steel lid containing holes. The MTP's were incubated in an incubator at 24 °C at 55 % relative humidity with 6 hrs of light. Assessment was made 5 days after infestation (% mortality on larvae). 6 replicates per concentration per product.
  - c. *Tetranychus urticae* (Preventative)
    - i. Bean plants were treated in a turntable sprayer (ARO 1-100 ml). After drying plants were infested with mites (mobile stages). The bean plants were incubated in a climatic room at 25 °C at 50 % relative humidity with 14 hrs of light. Assessment was made 8 days after infestation (number of eggs and % mortality of mites - all stages). Therefore a section of a leaf ( 48 mm diameter **punch size**) was punched and eggs were counted up to 50; if more than 50 classes were estimated and mites (nymphs and adults) were counted. 4 replicates per concentration per product.
3. **DOSE RESPONSE CURVES FOR ABAMECTIN (EC 018 = 1.8% / VERTIMEC) AND THIAMETHOXAM (WG 25 = 25% / ACTARA) WERE GENERATED WITH ALL THREE INSECTS – TABLES 1A-1E.**

**Table 1a & 1b :** Percent Mortality on *Heliothis virescens* (ovolarvicidal activity), eggs and 1<sup>st</sup> instars, when treated with Abamectin or Thiamethoxam

<b>TABLE 1a – Ovicidal Activity</b>		
Thiamethoxam (conc. in ppm)	Abamectin (conc. in ppm)	% Mortality
12.5	--	0
25	--	20
50	--	40
100	--	80
200	--	100
--	0.0125	0
--	0.025	0
--	0.05	28
--	0.1	30
--	0.2	58
--	0.4	75
--	0.8	65*

Water = 0

Check = 0

\* value not used for LC calculation

<b>TABLE 1b – Larvicidal Activity</b>		
Thiamethoxam (conc. in ppm)	Abamectin (conc. in ppm)	% Mortality
12.5	--	0
25	--	0
50	--	10
100	--	95
200	--	100
--	0.0125	0
--	0.025	5
--	0.05	28
--	0.1	53
--	0.2	80
--	0.4	88
--	0.8	90

Water = 0

Check = 0

**Table 1c:** Percent Mortality on *Plutella xylostella*, 2<sup>nd</sup> instars, when treated with Abamectin or Thiamethoxam

<b>TABLE 1c – Larvicidal Activity</b>		
Thiamethoxam	Abamectin	% Mortality

(conc. in ppm)	(conc. in ppm)	
12.5	--	30
25	--	23
50	--	38
100	--	35
200	--	45
--	0.0025	20
--	0.005	58
--	0.01	93
--	0.02	93
--	0.04	100
--	0.08	100

Water = 8

Check = 8

**Table 1d & 1e:**Percent Mortality on *Tetranychus urticae*, mixed population, when treated with Abamectin or Thiamethoxam

<b>TABLE 1d – Ovicidal Activity</b>		
Thiamethoxam (conc. in ppm)	Abamectin (conc. in ppm)	% Mortality
6.25	--	0
12.5	--	29
25	--	0
50	--	29
100	--	59
--	0.00156	0
--	0.00313	14
--	0.00625	14
--	0.0125	0
--	0.025	29
--	0.05	43
--	0.1	34

Water = 0

Check = 0

<b>TABLE 1e – % Mortality of Nymphs and Adults</b>		
Thiamethoxam (conc. in ppm)	Abamectin (conc. in ppm)	% Mortality
6.25	--	7
12.5	--	1
25	--	7
50	--	13
100	--	0

--	0.00156	5
--	0.00313	18
--	0.00625	0
--	0.0125	1
--	0.025	25
--	0.05	26
--	0.1	44

Water = 0

Check = 0

#### 4. RESULTS OF EXPOSURE TO VARIOUS MIXTURES OF ABAMECTIN AND THIAMETHOXAM

Tables 2a-2e give the percentage mortality of the mixtures (Found) together with the calculated (Expected) values based on the Colby Function.

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##### Colby Function (Expected Values)

$$E = (A + B) - \frac{A \times B}{100}$$

A = % protection of compound 1

B = % protection of compound 2

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**Tables 2a & 2b:** Percent Mortality on *Heliothis virescens*, eggs and 1<sup>st</sup> instars, when exposed to various mixtures of Abamectin (VERTIMEC EC 018) and Thiamethoxam (ACTARA; WG 25)

TABLE 2a – Ovicidal Activity on <i>Heliothis</i>					
Thiamethoxam (conc. in ppm)	Abamectin (conc. in ppm)	Ratio (A:T)	Found	Expected	Factor (F/E)
0.02875	--	--	0	--	--
0.0575	--	--	0	--	--
0.115	--	--	0	--	--
0.23	--	--	0	--	--
0.46	--	--	0	--	--
0.92	--	--	0	--	--
12.5	--	--	3	--	--
25	--	--	2	--	--
75	--	--	82	--	--
100	--	--	97	--	--
200	--	--	97	--	--
--	0.003215	--	0	--	--
--	0.00625	--	0	--	--
--	0.0125	--	3	--	--
--	0.025	--	3	--	--
--	0.05	--	22	--	--

--	0.1	--	42	--	--
--	0.2	--	77	--	--
--	0.8	--	83	--	--
0.02875	0.003125	1:9.2	0	0	--
0.02875	0.00625	1:4.6	0	0	--
0.02875	0.0125	1:2.3	0	3	0
0.0575	0.00625	1:9.2	0	0	--
0.0575	0.0125	1:4.6	0	3	0
0.0575	0.025	1:2.3	10	3	3.33
0.0575	0.05	1:1.15	63	22	2.86
0.115	0.0125	1:9.2	0	3	0
0.115	0.025	1:4.6	10	3	3.33
0.115	0.05	1:2.3	55	22	2.50
0.115	0.1	1:1.15	73	42	1.74
0.23	0.025	1:9.2	10	3	3.33
0.23	0.05	1:4.6	67	22	3.05
0.23	0.1	1:2.3	80	42	1.90
0.46	0.05	1:9.2	60	22	2.73
0.46	0.1	1:4.6	58	42	1.38
0.92	0.1	1:9.2	70	42	1.67
0.92	0.2	1:4.6	80	77	1.04

Water = 0

Check = 0

TABLE 2b – Larvicidal Activity on Heliothis					
Thiamethoxam (conc. in ppm)	Abamectin (conc. in ppm)	Ratio (A:T)	Found	Expected	Factor (F/E)
0.02875	--	--	0	--	--
0.0575	--	--	0	--	--
0.115	--	--	0	--	--
0.23	--	--	0	--	--
0.46	--	--	0	--	--
0.92	--	--	0	--	--
12.5	--	--	0	--	--
25	--	--	0	--	--
75	--	--	0	--	--
100	--	--	97	--	--
200	--	--	98	--	--
--	0.003215	--	0	--	--
--	0.00625	--	0	--	--
--	0.0125	--	0	--	--
--	0.025	--	7	--	--
--	0.05	--	55	--	--
--	0.1	--	95	--	--
--	0.2	--	100	--	--

--	0.8	--	100	--	--
0.02875	0.003125	1:9.2	0	0	--
0.02875	0.00625	1:4.6	0	0	--
0.02875	0.0125	1:2.3	0	0	--
0.0575	0.00625	1:9.2	0	0	--
0.0575	0.0125	1:4.6	0	0	--
0.0575	0.025	1:2.3	93	7	13.29
0.0575	0.05	1:1.15	85	55	1.55
0.115	0.0125	1:9.2	0	0	--
0.115	0.025	1:4.6	0	7	0
0.115	0.05	1:2.3	60	55	1.09
0.115	0.1	1:1.15	100	95	1.05
0.23	0.025	1:9.2	85	7	12.14
0.23	0.05	1:4.6	80	55	1.45
0.23	0.1	1:2.3	90	95	0.95
0.46	0.05	1:9.2	77	55	1.40
0.46	0.1	1:4.6	82	95	0.86
0.92	0.1	1:9.2	90	95	0.95
0.92	0.2	1:4.6	100	100	1.00

Water = 0

Check = 0

**Table 2c:** Percent Mortality on *Plutella xylostella*, 2<sup>nd</sup> instars, when exposed to various mixtures of Abamectin and Thiamethoxam

TABLE 2c – Larvicidal Activity on <i>Plutella xylostella</i>					
Thiamethoxam (conc. in ppm)	Abamectin (conc. in ppm)	Ratio (A:T)	Found	Expected	Factor (F/E)
0.002875	--	--	17	--	--
0.00575	--	--	12	--	--
0.0115	--	--	13	--	--
0.023	--	--	15	--	--
0.046	--	--	12	--	--
0.092	--	--	15	--	--
12.5	--	--	22	--	--
25	--	--	10	--	--
100	--	--	35	--	--
200	--	--	57	--	--
400	--	--	82	--	--
--	0.000313	--	20	--	--
--	0.000625	--	10	--	--
--	0.00125	--	10	--	--
--	0.0025	--	8	--	--
--	0.005	--	33	--	--
--	0.01	--	72	--	--

--	0.02	--	100	--	--
--	0.04	--	100	--	--
--	0.08	--	100	--	--
0.002875	0.000313	1:9.2	25	33.6	0.74
0.002875	0.000625	1:4.6	22	25.3	0.87
0.002875	0.00125	1:2.3	15	25.3	0.59
0.00575	0.000625	1:9.2	18	20.8	0.87
0.00575	0.00125	1:4.6	20	20.8	0.96
0.00575	0.0025	1:2.3	15	19.04	0.79
0.0115	0.00125	1:9.2	28	21.7	1.29
0.0115	0.0025	1:4.6	45	19.96	2.25
0.0115	0.005	1:2.3	60	41.71	1.44
0.0115	0.01	1:1.15	95	75.64	1.26
0.023	0.0025	1:9.2	40	21.8	1.83
0.023	0.005	1:4.6	60	43.05	1.39
0.023	0.01	1:2.3	85	76.2	1.12
0.023	0.02	1:1.15	100	100	1.00
0.046	0.005	1:9.2	43	41.04	1.05
0.046	0.01	1:4.6	92	75.36	1.22
0.046	0.02	1:2.3	100	100	1.00
0.092	0.01	1:9.2	92	76.2	1.21
0.092	0.02	1:4.6	98	100	0.98

Water = 12

Check = 12

**Table 2d & 2e:** Percent Mortality on *Tetranychus urticae*, mixed population, when exposed to various mixtures of Abamectin and Thiamethoxam.

TABLE 2d – Ovicidal Activity on <i>Tetranychus</i>					
Thiamethoxam (conc. in ppm)	Abamectin (conc. in ppm)	Ratio (A:T)	Found	Expected	Factor (F/E)
0.02875	--	--	45	--	--
0.0575	--	--	40	--	--
0.115	--	--	30	--	--
0.23	--	--	5	--	--
0.46	--	--	37	--	--
0.92	--	--	65	--	--
12.5	--	--	35	--	--
50	--	--	35	--	--
100	--	--	45	--	--
200	--	--	30	--	--
400	--	--	30	--	--
--	0.003215	--	0	--	--
--	0.00625	--	0	--	--

--	0.0125	--	0	--	--
--	0.025	--	40	--	--
--	0.05	--	50	--	--
--	0.1	--	20	--	--
--	0.2	--	64	--	--
--	0.4	--	98	--	--
--	0.8	--	96	--	--
0.02875	0.003125	1:9.2	25	45	0.56
0.02875	0.00625	1:4.6	40	45	0.89
0.02875	0.0125	1:2.3	52	45	1.16
0.0575	0.00625	1:9.2	25	40	0.63
0.0575	0.0125	1:4.6	45	40	1.13
0.0575	0.025	1:2.3	15	64	0.23
0.115	0.0125	1:9.2	20	30	0.67
0.115	0.025	1:4.6	20	58	0.34
0.115	0.05	1:2.3	20	65	0.31
0.115	0.1	1:1.15	25	44	0.57
0.23	0.025	1:9.2	42	43	0.98
0.23	0.05	1:4.6	63	52.5	1.20
0.23	0.1	1:2.3	66	24	2.75
0.23	0.2	1:1.15	69	65.8	1.05
0.46	0.05	1:9.2	35	68.5	0.51
0.46	0.1	1:4.6	50	49.6	1.01
0.46	0.2	1:2.3	97	77.32	1.25
0.92	0.1	1:9.2	81	72	1.13
0.92	0.2	1:4.6	87	87.4	1.00

Water = 0

Check = 0

TABLE 2e – Activity on Nymphs and Adults of Tetranychus					
Thiamethoxam (conc. in ppm)	Abamectin (conc. in ppm)	Ratio (A:T)	Found	Expected	Factor (F/E)
0.02875	--	--	39	--	--
0.0575	--	--	40	--	--
0.115	--	--	36	--	--
0.23	--	--	30	--	--
0.46	--	--	44	--	--
0.92	--	--	52	--	--
12.5	--	--	58	--	--
50	--	--	57	--	--
100	--	--	31	--	--
200	--	--	41	--	--
400	--	--	52	--	--
--	0.003215	--	0	--	--
--	0.00625	--	0	--	--

--	0.0125	--	0	--	--
--	0.025	--	0	--	--
--	0.05	--	35	--	--
--	0.1	--	51	--	--
--	0.2	--	66	--	--
--	0.4	--	89	--	--
--	0.8	--	97	--	--
0.02875	0.003125	1:9.2	48	39	1.23
0.02875	0.00625	1:4.6	62	39	1.59
0.02875	0.0125	1:2.3	64	39	1.64
0.0575	0.00625	1:9.2	53	40	1.33
0.0575	0.0125	1:4.6	25	40	0.63
0.0575	0.025	1:2.3	43	40	1.08
0.115	0.0125	1:9.2	0	36	0
0.115	0.025	1:4.6	21	36	0.58
0.115	0.05	1:2.3	9	58.4	0.15
0.115	0.1	1:1.15	35	68.64	0.51
0.23	0.025	1:9.2	0	30	0
0.23	0.05	1:4.6	0	54.5	0
0.23	0.1	1:2.3	38	65.7	0.58
0.23	0.2	1:1.15	33	76.2	0.43
0.46	0.05	1:9.2	0	63.6	0
0.46	0.1	1:4.6	35	72.56	0.48
0.46	0.2	1:2.3	33	80.96	0.41
0.92	0.1	1:9.2	32	76.48	0.42
0.92	0.2	1:4.6	54	83.68	0.65

Water = 0

Check = 0

**CONCLUSIONS**

5. Clear, unexpected synergism of the mixture, Abamectin and Thiamethoxam, was found on *Heliothis virescens* (eggs and larvae) at different ratios (see Tables 2a and 2b). For example, an ovicidal activity of 67% was found when mixing 0.23 ppm of Thiamethoxam with 0.05 ppm of Abamectin (see Table 2a). The expected activity of this mixture was 22%; this means an increase on activity of 45%.
6. Another unexpected synergism of the mixture, Abamectin and Thiamethoxam, was found on *Plutella xlyostella* (see Table 2c). For example, a larvicidal activity of 40% was found when mixing 0.023 ppm of Thiamethoxam with 0.0025 ppm of Abamectin (see Table 2c). The

expected activity of this mixture was 21.8%; this means an increase on activity of 18.2%.

Also, a larvicidal activity of 45% was found when mixing 0.0115 ppm of Thiamethoxam with 0.0025 ppm of Abamectin (see Table 2c). The expected activity of this mixture was 19.96%; this means an increase on activity of 25.04%.

**FINAL STATEMENT**

I, Elke Hillesheim, declare further that all statements made herein of personal knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of this application or any patent issuing thereon.

Signed this 2nd day of June 2008

*E. Hillesheim*

ELKE HILLESHEIM